1981

The relationship of serum thyroxine to the reproductive capabilities of prairie deermice (Peromyscus maniculatus bairdi) from laboratory populations

Edgar David Peebles
College of William & Mary - Arts & Sciences

THE RELATIONSHIP OF SERUM THYROXINE TO THE REPRODUCTIVE CAPABILITIES OF PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDI*) FROM LABORATORY POPULATIONS

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

E. David Peebles, III

1981

APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts

E. David Peebles, III
Author

Approved, December 1981.

Eric L. Bradley
Eric L. Bradley

C. Richard Terman
C. Richard Terman

Robert E. L. Black
Robert E. L. Black

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I express deep appreciation to Dr. Eric Bradley, my graduate advisor, for his patient guidance and instructive assistance. Also, I thank Dr. C. Richard Terman and Dr. Robert Black for serving on my graduate committee. I am indebted to Mrs. Jewel Thomas for her expert technical assistance and Mrs. Ruby Thomas Peebles for typing the manuscripts. A portion of this investigation was supported by NIH research grant HD 08906 from the National Institute of Child Health and Human Development.

LIST OF TABLES AND FIGURES

ABSTRACT

The objective of this study was to examine the relationship of serum thyroxine to the reproductive capabilities of laboratory populations of prairie deermice. This was in part accomplished by evaluating the serum thyroxine concentrations and reproductive organ weights in population and control males and females.

Within the age group studied, serum levels of thyroxine in population animals of both sexes had no correlation with reproductive organ weight, reproductive status, or age.

An evaluation of serum thyroxine concentration at various time periods in bisexually paired controls during part of the day showed that thyroxine levels were not both low and stable simultaneously for both sexes. The period between two and one hours prior to the onset of darkness was, therefore, selected for subsequent blood sampling because previous studies had been done at this time.

It was shown that population females tended to have lower mean thyroxine concentrations in comparison to control females, and population males had significantly lower mean levels of thyroxine than their controls. In the same study, the reproductive organ and body weights of population animals of both sexes were significantly smaller than bisexually paired controls, indicating drastic reproductive inhibition in population animals.

The administration of physiological doses of thyroxine to reproductively inhibited population male deermice appeared to produce an increase in spermiogenesis. Further investigations with sham injected controls are needed to clarify the significance of these findings.

The data indicate that lower serum levels of thyroxine in reproductively inhibited population deermice may be a factor contributing to their reproductive inhibition. Additional work is required to determine if the thyroid is a "primary" contributor to reproductive inhibition.

THE RELATIONSHIP OF SERUM THYROXINE TO THE REPRODUCTIVE CAPABILITIES OF PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDI*) FROM LABORATORY POPULATIONS

INTRODUCTION

Natural population levels of the prairie deermouse, Peromyscus maniculatus bairdi, have been reported (Terman, 1966) to have lower average ranges of numerical fluctuation and longer fluctuation times than most of the other rodent species studied in the "North American Census of Small Mammals." Terman (1966) has suggested that this may indicate that the prairie deermouse has a greater sensitivity to the factors that influence their population growth. In the laboratory, populations of P. maniculatus bairdi control their growth at markedly variable numerical levels in different populations maintained under similar environmental conditions and with surplus food and water (Terman, 1965). Growth of laboratory populations of prairie deermice is known to be controlled by failure of young to survive and/or by cessation of reproduction (Terman, 1965). In populations that are controlled by these processes, approximately 94% of the females born into the populations, which are a minimum of 90 days of age, do not reproduce and the mean weights of the reproductive organs of both males and females born into the populations are significantly smaller than bisexually-paired controls (Terman, 1965, 1969, 1973).

Further, in a study by Christian (1955) it was found that seminal vesicle and preputial gland weights of Peromyscus leucopus decreased with increasing population size; this species is also known to have a low average natural population size and range of fluctuation (Terman, 1966). Partial or complete inhibition of reproduction has been found to be a common correlate of increased density in a number of mammalian populations (Christian, 1978).

It has been established that endocrine feedback responses usually accompany increased density in populations of small mammals including P. maniculatus bairdi, and that these can limit population growth (Christian, 1978, 1980). Increased population density may be associated with an increase in stressful social interactions and strife, causing various changes in secretions by the pituitary and adrenal glands which may become inhibitory to reproduction (Christian, 1960, 1971a,b, 1978, 1980). Stressful situations brought on by such things as crowding, immobilization, anxiety, fear, or movement to a strange environment have been classified as chronic stress (Christian, 1978), while acute stress denotes a sudden short-term traumatic event. Chronic stress may be less extreme than acute stress, but it is constant and prolonged. Stress resulting from increased numbers and aggressive interactions in dense populations may be a major form of chronic stress and may operate in reproductively-inhibited population deermice. Studies on numerous

mammals have shown that both chronic and acute stress can induce changes in the secretions of the hypothalamus, anterior pituitary, adrenals, and thyroid (Chan, et al., 1978; Christian, 1980; Döhler, et al., 1977; Eisenbud, 1975; Fleischer, et al., 1978; Fortier, et al., 1970; Knigge, et al., 1959; Kruhlich, et al., 1974; Langer and Lichardus, 1969; Reichlin, 1966).

Chronic stress seems to be inhibitory to reproductive function by its marked suppression of luteinizing hormone (LH), follicle stimulating hormone (FSH), and growth hormone (GH) secretion in rats (Christian, 1978, 1980; Negro-Vilar, et al., 1971). Acute stress in the rat due to handling has been shown to affect the secretions of LH, FSH, GH, and prolactin (PRL) differently. FSH secretion is less susceptible to stress than LH secretion, but when elicited, both show an initial stimulatory phase and then a more prolonged inhibitory phase. PRL release is enhanced and GH is inhibited with the same types of acute stress (Kruhlich, et al., 1974). Similarly, Döhler, et al., (1977) found that in the rat PRL concentration increased five minutes after disturbance, FSH concentration initially rose 10 minutes later and then fell at 60 minutes after the disturbance, but LH concentration underwent only minor fluctuations. Dunn, et al., (1972) observed that acute stress may also abolish the circadian rhythm of serum LH levels in adult male rats.

Increased density or social strife has been found to be accompanied by increased secretion of corticotrophin (ACTH) by the anterior pituitary and of some adrenal steroids in various mammals (Christian, 1971a), and recent studies summarized by Christian (1978) also indicate that chronic stress may result in increased adrenocortical secretions. Knigge, et al., (1959) found that immobilization (chronic) stress resulted in the increased secretion of corticosterone in rats, and that there was an initial biphasic response (elevation, then drop) followed by a high sustained level of hormone secretion. Studies showing adrenocortical hypertrophy occurring in populations of increasing size support the major importance of stress-induced hyperfunction of the pituitary-adrenal axis in inhibiting reproductive function (Christian, 1960; Christian, et al., 1960; Christian and Davis, 1964). ACTH will inhibit reproduction in several strains of intact, immature mice (Mus) (Christian, 1964, 1971b), and in P. maniculatus bairdi ACTH may inhibit sexual maturation of intact mice and induce reproductive regression in adults (Christian, 1971a). Christian (1964) has suggested that ACTH may inhibit reproduction by directly suppressing gonadotrophin secretion or by indirectly increasing adrenal steroid production.

The pituitary-thyroid axis may be affected by stress differently than the pituitary-adrenal axis. There are, however, conflicting reports in the literature on the effects

of chronic stress on thyroid function. In wild and laboratory rabbits, the stress of immobilization for 24 to 72 hours led to an inhibition of thyroidal I^{131} release (Brown-Grant, et al., 1954b). The restraint-induced thyroid inhibition was eliminated by sectioning the pituitary stalk, thus indicating the participation of the hypothalamus in the stress response (Brown-Grant, et al., 1957). On the other hand, evidence that chronic emotional stress can increase thyroid function was given by Mason, et al., (1961) who found that after a period of adversive conditioning in the monkey, plasma protein-bound iodine increased. Volpé, et al., (1960) conversely reports that the effects of emotional tension and anxiety in humans failed to produce any significant fluctuations in their serum protein-bound iodine levels.

There are also contradictory reports on the response of the thyroid and of thyrotrophin (TSH) release to acute stress. In a study by Döhler, et al., (1977) serum thyroxine fluctuated insignificantly and triiodothyronine was found to rise with corticosterone after disturbance stress in rats, while Chan, et al., (1978) reports a sharp early rise in total serum thyroxine after surgical stress in animals. Langer and Lichardus (1969), however, found that during short-term stress in rats, the secretion of TSH and thyroid function were decreased simultaneously with increased ACTH secretion, and Dewhurst, et al., (1968) states that, "There is thus little good evidence... that thyroid stimulation

follows acute stress." Elevated serum TSH levels after transport stress have been observed by Döhler, et al., (1977), and Wong, et al., (1977); but, Ducommun, et al., (1966) observed decreased TSH levels after transport stress. Fenske and Wuttke (1977) found no evidence of influence. Although reports on the effects of stress on thyroid function are inconsistent, the thyroid is possibly influenced by stress; this influence may be significant in laboratory populations of deermice.

There are distinctive interactions between the pituitary, adrenals, and thyroid; their influence on one another is considered to be an important factor in reproductive function. Ooka-Souda, et al., (1979) report that adrenalectomy reduced plasma corticosterone levels to almost zero in male rats and, concurrently, decreased plasma triiodothyronine and thyroxine levels. Other workers, however, have proposed that increased pituitary-adrenocortical activity may be inhibitory to the thyroid (Christian, 1963) and that a reciprocal relationship between ACTH and TSH secretions exists (Retiene, et al., 1968). Reineke and Soliman (1953) give evidence that thyroidal I^{131} uptake is depressed by exogenous progesterone in rats, and in man, ACTH and hydrocortisone doses were found to inhibit the release of TSH and thyroid hormone (Levin and Daughaday, 1955; Nicoloff, et al., 1970). Cortisone administration was also shown to reduce the thyroid's rate of release of radio-

iodine (Brown-Grant, 1955), while exogenous dexamethasone (synthetic glucocorticoid) reduced plasma TSH concentration in rats (Wilber and Utiger, 1969). These data suggest that stress may be capable of indirectly inhibiting thyroidal activity via increased activity of the pituitary-adrenal axis.

Studies on the effects of the thyroid on the adrenals indicate an influence which is opposite to that of the adrenals on the thyroid. Many investigations conclude that hypothyroidism, produced either by thyroidectomy or antithyroid drugs, causes adrenal atrophy (Fortier, et al., 1970; Gaunt, et al., 1970; Peterson, 1958). In hypothyroid patients between 5 and 17 years of age, cortisol secretion is decreased, while it is increased in hyperthyroid patients (Kenny, et al., 1967). Thyroid dysfunction has also been found to alter the circadian rhythm of cortisol secretion in humans (Martin, et al., 1963).

A reduction in the secretion and plasma levels of gonadotrophins have likewise been associated with hypothyroidism (Buchanan, et al., 1977; Chu, 1944a; Hagino, 1971; Larochelle and Freeman, 1974; Reineke, et al., 1941). Propylthiouracil (PTU) treatment was found to induce a marked decline in pituitary weight and LH content, as well as ovarian weight in rats (Hwang, et al., 1974). In addition, Dunn, et al., (1976) showed that the daily rhythmic release of LH and PRL in rats was altered by thyroidectomy. There are reports, however, that anterior pituitary

gonadotrophin content was unchanged in thyroidectomized female (Smith and Engle, 1930) and male (Smelser, 1934) rats, and that serum levels of LH and FSH were not altered by mild maternal hypothyroidism in rats (Hendrich, et al., 1976). Chu (1944b), on the other hand, found higher FSH production and diminished LH levels in the hypophysis of thyroidectomized rabbits.

Although there are variable results in the literature concerning the effects of thyroid dysfunction on reproduction, and the means by which the thyroid affects reproductive function is controversial, it is generally established that the thyroid is involved in normal reproductive function and impairment of its activity may be inhibitory to reproduction (Bruce and Sloviter, 1957; Hoar, et al., 1957; Maqsood, 1950; Parrott, et al., 1960; Peterson, et al., 1952). Conflicting reports on the influence of the thyroid on reproductive function, however, may in large part be due to differences in environmental factors, the methods by which thyroid dysfunction is produced, and the age, species, and stage of sexual development of the animals used. Thyroid deficiency has been found to disrupt reproductive function in man and experimental animals in many ways. The inhibitory effects of hypothyroidism include processes ranging from the arrest of sexual activity and development in males and females to the decline in the sizes and numbers of young born to hypothyroid females.

Indications in the literature that stress and pituitary-adrenal hyperactivity adversely affect thyroid activity and reproductive function, and that thyroid insufficiency may alter gonadotrophin secretion and inhibit reproductive function, suggest that serum thyroxine may influence the reproductive capabilities of laboratory populations of deermice. Endocrine studies on Peromyscus maniculatus bairdi have been limited to progesterone (Albertson, et al., 1975), ACTH (Coppes, 1980), corticosterone (Sung, et al., 1977), testosterone (Bradley and Terman, 1981c), LH, and FSH (Bradley and Terman, 1981a). To determine if the thyroid might be a factor contributing to the reproductive inhibition of laboratory populations of deermice, a series of experiments were undertaken which included the evaluation of serum thyroxine levels and reproductive organ weights of reproductively-inhibited population animals and their respective controls.

MATERIALS AND METHODS

Animal Maintenance

The animals used in this study were prairie deermice (Peromyscus maniculatus bairdi) obtained from a laboratory colony in which no sibling matings were allowed. Colony animals were kept in 12.5 x 27 x 14.5 cm plastic, wire-top cages which were illuminated daily by bright light (four 40W flourescent tubes) for 14 hours (0700-2100 hrs) and in darkness for 10 hours. All control and population animals were maintained on food (D&G Lab Diet and Wayne Laboratory Animal Diet) and tap water in excess of utilization. Laboratory temperatures were maintained between 21° and 30° C.

Experimental populations were founded by four pairs of reproductively proven mice with males and females from different litters. Founding animals were paired at between 63 and 102 days of age and were then left paired between 19 and 39 days before being released into the population enclosures. The first litter of those females which were pregnant upon introduction into the enclosures was discarded so that only young produced within the population context would be part of the population. All populations were at least 8½ months old before any animals were taken from them for study. Population enclosures consisted of corrugated

aluminum walls, standing within a circular stainless steel base 1.5 m in diameter. The floor area was covered with woodshavings and eight plastic nest boxes were provided in each enclosure (Terman, 1969, 1973).

Collection of Tissues

Blood samples were taken from control and population animals by using ether anesthesia and then cutting the dorsal abdominal aorta at the level of the kidney via ventral abdominal incision. All animals were sacrificed in less than one hour after initial disturbance. Based on findings by Döhler, et al., (1977), that serum levels of triiodothyronine in rats did not fluctuate significantly until 60 minutes after disturbance while thyroxine never fluctuated significantly, it was assumed that limiting the sacrifice time to within one hour after disturbance was satisfactory. Sera, which were obtained by centrifuging blood samples for three minutes at 9,000 x g, were stored below -30°C until assayed for thyroxine.

All testes, seminal vesicles, ovaries, and uteri were fixed in 10% buffered formalin for at least 72 hours and were cleaned of surrounding tissue before being weighed or examined. The wet weights of the reproductive organs were measured on a Cahn electrobalance DTL in connection with a Wang 700 programmable calculator.

Preliminary Investigations

A preliminary test was undertaken to establish whether there might be significant differences in serum thyroxine levels between laboratory population animals and animals maintained outside of populations in a laboratory colony. Four males and four females (aged 472 to 1195 days) were selected from a population without regard to age or reproductive status. The photoperiod for the population was 14 hours of bright light from 2200-1200 hours. This was followed by ½ hour of total darkness, 9 hours of dim light, and ended with ½ hour of darkness. This "dim" period permitted behavioral observations on this population for an earlier study that is not reported here. Four female and four male control animals were also taken at approximately 120 days of age directly from the laboratory colony described earlier. These animals had been kept in cages with sibs of the same sex since weaning. Serum samples were taken from all of these animals at a time relative to their photoperiod light-to-dark transition equal to two hours before the onset of the dark period.

Sera from the control and population animals were assayed for thyroxine concentration and the mean values were compared. The body weights of the control and population animals were also measured and compared.

Advantage was also taken of the opportunity to evaluate serum thyroxine concentrations in all of the animals (13

males, 15 females; aged 542 to 1498 days) remaining in the population that had been used in the first Preliminary Investigation. Specifically, the intention was to determine if there were any correlations between serum thyroxine concentrations and either reproductive organ weight, reproductive status, or age. Sera were taken from each of these animals in the interval between 1000 to 1100 hours, which corresponds to an interval between one and two hours prior to the light-to-dark transition (darkness: 1200 to 2200 hrs, daily, as described above) for these animals. The sera taken from these animals were analyzed for thyroxine concentration and their reproductive organs (testes, seminal vesicles, ovaries and uteri) were removed and weighed.

Main Studies

EXPERIMENT ONE: Because serum thyroxine concentration is known to change with the time of day in most species, it was important to establish the nature of such a pattern in deermice for that part of the day that was most suitable for sampling. In order to study this, control animals from different litters were placed as bisexual pairs into "no-contact" cages. These 12 x 26 x 24 cm wire mesh cages were divided by a partition composed of two layers of wire mesh two cm apart, which allowed the exchange of visual and olfactory cues between the paired animals but prevented physical contact (Albertson, et al., 1975). Mice were paired

at 94 ± 8 days of age, were kept on a 14-hour (0800-2200 hrs) bright light and a 10-hour dark cycle for 26 ± 7 days, and then sacrificed. Five pairs of animals were sacrificed at 1800, 1900, 2000, 2130, 2400, and 0200 hours.

Sera were collected for all time groups and assayed for thyroxine concentration. The mean serum thyroxine levels at each of the time periods were calculated for each sex and differences between their values were evaluated.

EXPERIMENT TWO: This study was undertaken to establish the degree of difference in serum thyroxine levels and reproductive organ and body weights between reproductively-inhibited population animals and controls maintained as pairs. Population animals were sacrificed at 123 ± 3 days of age and control animals were paired bisexually at 82 ± 2 days of age, then placed in "no-contact" cages for 30 ± 2 days and subsequently sacrificed at 120 ± 2 days of age. Both control and population mice were maintained daily on 14 hours of bright light (from 2200-1200 hrs) and 10 hours of darkness. At the time of population sampling, a 40W long-wave uv lamp was used to identify mice which had been marked at least 24 hours earlier with a non-toxic uv-sensitive dye (Raytech Industries). All population and control animals were sacrificed at between 1000 and 1100 hours and sera were collected and stored frozen until analyzed for thyroxine concentration. The reproductive organs (testes, seminal vesicles, uteri, and ovaries) from the 18

pairs of control and 22 pairs of population mice were fixed in formalin and weighed as described earlier. The body weights of the control and population animals were also measured and their mean values compared.

Radioimmunoassay Methods

Total (free and protein-bound) serum thyroxine concentrations were determined by a competitive binding technique prescribed by the T_4RIA (PEG) Diagnostic Kit sold by Abbott Laboratories (Chicago, Illinois). This method utilized I^{125} labeled thyroxine and thyroxine antiserum (rabbit), which contained 8-anilino-1-napthalenesulfonic acid (ANS) to extract bound thyroxine from its binding proteins and to prevent further binding of the thyroxine by these proteins. I^{125}-thyroxine and thyroxine antiserum were added respectively to standard solutions containing known amounts of thyroxine and to experimental samples containing unknown amounts of thyroxine. The mixtures were incubated at room temperature for one hour, allowing the labeled and unlabeled thyroxine fractions to bind to antiserum in proportion to their concentrations. Antiserum-thyroxine complexes were precipitated and separated from unbound thyroxine by polyethylene glycol (PEG) and centrifugation (1,000 x g).

Supernatants in each assay tube were decanted and the net radioactivity of the I^{125}-thyroxine bound in the complexes were counted on a Beckman Biogamma II counter.

Radioactivity was counted to a 2% error in all Experiments. The amount of I^{125}-thyroxine bound in the complexes and their radioactivities were inversely proportional to the amount of non-radioactive thyroxine in the standard or experimental solution. A standard curve plotting percent bound I^{125}-thyroxine versus the corresponding unlabeled thyroxine concentration in standards was used to determine the concentration of serum thyroxine in experimental samples.

Statistical Methods

Comparisons of serum thyroxine concentration, and body and reproductive organ weights in control and population animals were made utilizing the Student's t-test or Mann-Whitney U-test. The Student's t-test was used to compare experimental groups if an F-test showed a lack of heterogeneity of variances. If the variances were heterogenous, comparisons were made with the Mann-Whitney U-test. Linear regression analyses were run to determine if correlations existed between the thyroxine level and age, reproductive status and the reproductive organ and body weights of population mice. A probability of less than 0.05 was considered significant in all cases.

RESULTS

Preliminary Investigations

The mean serum thyroxine concentrations of the four males and the four females taken from the population were both significantly ($P < 0.028$) lower than the corresponding mean values from the four male and the four female controls. There were no significant differences in the mean thyroxine values between the sexes in either group. The mean body weights of both males and females from the population were also significantly ($P < 0.05$) smaller than the corresponding control animals (Appendix A). These data strongly suggested that a more extensive investigation of serum thyroxine concentrations as an index of thyroid function was justified.

The linear regression analyses of the data for serum thyroxine compared with weights of the respective reproductive organs (ovaries, uteri, testes, seminal vesicles) from both males and females taken from a single population showed no significant correlations in any comparisons (see Appendices B and C). There were, also, no significant correlations between age and body weight or body weight and reproductive organ weight. Only in population males was there a tendency ($P < 0.1$) toward a negative correlation of

age and serum thyroxine concentration. Finally, no correlations were found between the values assigned to the frequency of occurrence of vaginal perforation or to pregnancy and lactation in females, or for the frequency of occurrence of scrotal testes in males and the respective serum thyroxine concentration.

Main Studies

EXPERIMENT ONE: The values obtained for serum thyroxine concentrations at selected times before and after the light-to-dark transition revealed several interesting points. There was a general reduction from the high serum thyroxine concentration observed in both sexes four hours before the onset of darkness to the lowest values observed at three hours before dark in females, and two hours before dark in males. Following the low point, serum thyroxine concentration in males rose significantly ($P < 0.05$) to elevated values 30 minutes before darkness and these values remained elevated after the change to dark (cf. Table 1 and Figure 1). The values from females revealed a different pattern of response in that there was a transient but not significant ($P < 0.1$) rise in serum thyroxine concentration between three and two hours prior to dark, but by 30 minutes prior to dark the value was again low and remained so throughout the dark period (cf. Table 1 and Figure 1). The mean serum thyroxine concentration in males was higher than

females except at two hours prior to darkness. These values, however, were significantly higher only at 30 minutes prior to and four hours after dark (Table 1).

These data suggest that within the period evaluated there is not a time when the serum thyroxine values are simultaneously low and stable for both sexes. It was, therefore, decided to sample animals for subsequent work at between two and up until one hour prior to the light-to-dark transition because earlier work had been done at this time, in this study and in other hormone studies on P. maniculatus bairdi (Bradley and Terman, 1981a).

EXPERIMENT TWO: In this Experiment, 22 male and 22 female reproductively-inhibited population derived animals were compared with 18 control pairs. It was found that population females tended ($P < 0.1$) to have a lower mean serum thyroxine concentration in comparison with control females (Table 2), and that the mean thyroxine value for population males was significantly ($P < 0.03$) lower relative to control males (Table 3). The mean reproductive organ weights (testes, seminal vesicles, ovaries, and uteri) in population animals of both sexes were highly significantly ($P < 0.001$) smaller than the respective weights of control animals. The mean body weights of both males and females from populations were significantly ($P < 0.01$ and $P < 0.001$, respectively) smaller than the corresponding control animals (cf. Tables 2 and 3).

TABLE 1

Serum thyroxine concentrations at various times relative to the light-to-dark photoperiod transition in male and female control pairs. Values are mean ± SEM.

	4 hrs before dark (1800 hrs)	3 hrs before dark (1900 hrs)	2 hrs before dark (2000 hrs)	30 mins before dark (2130 hrs)	2 hrs after dark (2400 hrs)	4 hrs after dark (0200 hrs)
Males (n=5)	4.6 ±0.70	3.5 ±0.43	2.4 ±0.44	4.2 ±0.54	4.5 ±0.19	3.7 ±0.60
Females (n=5)	$4.2^{n.s.}$ ±0.58	2.1* ±0.37	$3.2^{n.s.}$ ±0.54	2.5*** ±0.33	2.6* ±0.36	2.4** ±0.28

*P< 0.1, **P< 0.05, ***P< 0.01 and n.s. = not significant with respect to the corresponding value for males.

TABLE 2

Serum thyroxine, body weight, and ovary and uterus weights in control and population females. Values are mean ± SEM.

Treatments	Serum thyroxine (ug/100 ml)	Body weight (g)	Ovary weight (mg)	Uterus weight (mg)
Control Females (n=18)	2.47 ±0.16	16.25 ±0.48	17.77 ±1.41	47.77 ±4.61
Population Females (n=22)	2.08* ±0.12	12.38** ±0.35	5.34** ±0.52	9.84** ±2.25

*$P < 0.1$ and **$P < 0.001$ with respect to the corresponding control value.

TABLE 3

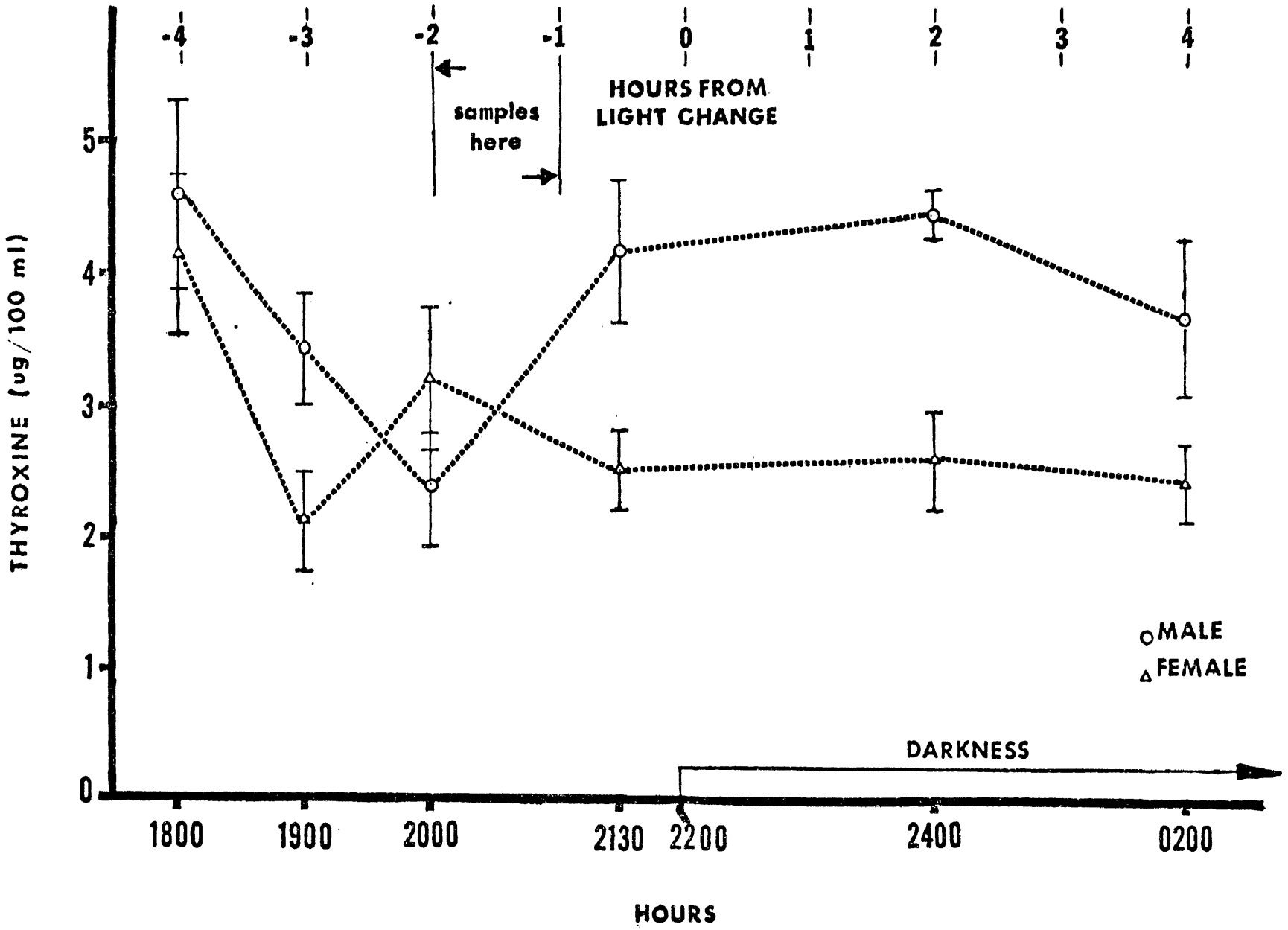
Serum thyroxine, body weight, and testis and seminal vesicle weights in control and population males. Values are mean ± SEM.

Treatments	Serum thyroxine (ug/100 ml)	Body weight (g)	Testis weight (mg)	Seminal vesicle weight (mg)
Control Males (n=18)	3.09 ±0.24	17.08 ±0.40	299.33 ±20.28	145.52 ±14.76
Population Males (n=22)	2.41* ±0.12	14.73** ±0.63	92.78*** ±13.50	12.81*** ±4.46

*P < 0.03, **P < 0.01, and ***P < 0.001.

FIGURE

Exp. One - Serum thyroxine concentrations at various times in male and female control pairs, showing the fluctuations in their serum thyroxine between four hours before and four hours after the light-to-dark photoperiod transition.


-4
-3
-2
-1
0
1
2
3
4
HOURS FROM LIGHT CHANGE
samples here
THYROXINE (ug/100 ml)
5
4
3
2
1
0
MALE
FEMALE
DARKNESS
1800
1900
2000
2130
2200
2400
0200
HOURS

DISCUSSION

Terman (1969) found no significant differences between the mean body weights of control and population deermice in either sex. However, later studies using different control caging arrangements (Bradley and Terman, 1981a; Sung, et al., 1977) revealed that the mean body weights of both male and female mice taken from populations were significantly less than their respective controls. Similarly, in another study, Bradley and Terman (1981b) reported that male population mice were significantly smaller in body weight than controls, and that population females also tended to be lighter than their controls, but not significantly. The mean body weights of both male and female population mice in the present study were also found to be significantly lighter than the corresponding controls. In the second Preliminary Investigation, body weight and age were found not to correlate significantly over the restricted age range of between 542 and 1498 days of age. Apparently, the body weight-age correlations which clearly exist in young growing animals do not persist in old adults.

It is tempting to speculate that the smaller body weights of population-derived animals may be directly related to the lower thyroxine levels found in the population animals

of this study. Most vertebrates cannot attain adult size in the absence of normal thyroid secretions throughout their development (Turner and Bagnara, 1976). Thyroid hormone is not only necessary for synergistic activity with growth hormone (GH) in promoting normal growth, but it may also be necessary for proper GH secretion. GH content of the anterior pituitary and serum in rats was found to be negatively affected by thyroidectomy, and the treatment of pregnant and lactating rats with propylthiouracil (PTU) resulted in the inhibition of gonadal development and lower serum levels of GH and PRL in their pups (Coiro, et al., 1979; Contopoulos, et al., 1958; Kikuyama, et al., 1974).

It is important to recognize that many factors acting alone or in conjunction with thyroid hormone could be responsible for reduced body weight in population animals. For example, body weight in population mice might be inhibited by various secretions of the adrenal cortex. Under stressful stimuli, the release of GH has been reported to be inhibited in proportion to the degree of stimulation of the adrenal cortex (Christian, 1978; Takahashi, et al., 1971; Terry, et al., 1976). In addition, glucocorticoids alone are known to inhibit growth (Christian, 1978). Thus, density-related stress among population deermice may be inhibitory to growth through both the pituitary-thyroid and the pituitary-adrenal axes.

The mean reproductive organ weights (testes, seminal vesicles, uteri, and ovaries) in population deermice of both

sexes have been consistently found to be significantly smaller than their respective bisexually paired controls. (Albertson, et al., 1975; Bradley and Terman, 1981a; Sung, et al., 1977; Terman, 1969, 1973). In Experiment Two of this study, the mean reproductive organ weights of reproductively inhibited population animals of both sexes were also significantly ($P < 0.001$) smaller than their respective controls of the same age. However, there was no correlation between the body weight and reproductive organ weight for the population males and females sampled in the second Preliminary Investigation. One might conclude from these data that within the range of ages (542 to 1498 days) studied body and reproductive organ weights might be similarly affected by environmental influences, but that body weight does not govern reproductive organ weight.

The main objective of this study was to examine serum thyroxine concentration in deermice and to define the relationship between these concentrations and reproductive condition. Serum thyroxine was analyzed instead of triiodothyronine because of its intrinsic hormone activity and demonstrated value in the diagnosis of hypothyroidism in other mammalian species (Chopra, et al., 1973; Kirkegaard, et al., 1974; McConnon, et al., 1971).

Recent studies show that thyroid function and serum thyroxine concentrations fluctuate with a circadian rhythm (Jordan, et al., 1980; Romanov and Stepanova, 1975). In male rats, Jordan, et al., (1980) found significant daily

variations in thyroxine, with zenith and nadir levels occurring during mid-afternoon and during nighttime, respectively. Likewise, Pallardo, et al., (1976) reports significant diurnal variations in the thyroid/serum iodide ratios and the absolute uptake of iodide in male rats. Peaks in these values, however, occurred during the night rather than the day. In the male rabbit, highly significant diurnal variations in protein-bound iodine were found, with a high occurring from mid-morning to noon and a low appearing at about 2000 hours (Laird and Fox, 1970). DeCostre, et al., (1971) described the occurrence of parallel diurnal variations in total thyroxine and I^{131} labelled thyroxine (PBI^{131}), with each having maximum plasma concentrations between 1000 and 1400 hours, and a minimum around 0200 hours. Weeke and Gunderson (1978) found a tendency for a synchronous diurnal rhythm of free thyroxine and serum TSH in humans, but the largest daily variation of 7% was not statistically significant.

Because a diurnal serum thyroxine rhythm was likely to exist in P. maniculatus bairdi, Experiment One was undertaken to determine a daily time period, within four hours before and after the light-to-dark transition, when thyroxine concentrations were most stable. Within the interval studied, however, there was not a time period when serum thyroxine concentrations were simultaneously low and stable for both males and females (Figure 1). It was, therefore, decided to sample animals in later experiments within the interval between the second and first hour before the light-to-dark transition.

Hence, animals in Experiment Two were sampled between 1000 and 1100 hours, with the change to dark at 1200 hours. It may be of importance to note that the elevated serum thyroxine concentrations in males, reached 30 minutes prior to the light-to-dark transition, remained consistently higher than the corresponding values in females for at least four hours into the dark period. Such higher serum thyroxine levels in males could be related to their increased activity and aggressiveness compared with females during the dark period.

The relationship of age to serum thyroxine concentration in population animals was examined in the second Preliminary Investigation. No significant correlation was found between the ages (700 to 1498 days old) of population males or the ages (542 to 1498 days old) of population females and their respective thyroxine levels (Appendices B and C). There tended ($P < 0.1$), however, to be a negative correlation in males. Although this observation on a restricted sample may indicate that there is decreasing serum thyroxine concentration with increasing age, a much broader range of ages and reproductive conditions must be examined to demonstrate this conclusively.

Many studies on other mammalian species suggest that there is an overall decline in thyroidal function with age in both sexes, but that it is not necessarily linear, with fluctuations occurring at certain periods in the aging process. In female rats, Chen and Walfish (1978) found that the older animals had lower total and free thyroxine as well as lower

free triiodothyronine concentrations. Azizi (1979) reported that as young adult male rats (from 30 to 150 days of age) grow older, their intrinsic thyroid functions decline and their serum thyroxine levels fall by 50%, while the serum triiodothyronine levels remain unchanged. Conversely, in a study by Heinen, et al., (1981) serum thyroxine levels were shown to decrease insignificantly with old age in rats, whereas, serum triiodothyronine was significantly lower in the older than in the younger rats. Likewise, in humans triiodothyronine was found to decrease progressively and to an equal extent with age in both sexes (Rubenstein, et al., 1973). In the postnatal development of the rat, the first two days after birth and the third week have been found to be periods of increased activity in the pituitary-thyroid system, and during the fifth week the decline in thyroidal I^{131} uptake to adult values took place (Samel, 1968). Similarly, Vigouroux (1976) found that maximum plasma concentrations of thyroxine occurred 16 days after birth and that thyroxine secretion was more intense prior to than after weaning during postnatal development in the rat. In humans, Hollis (1968) indicated that I^{131} 24-hour uptake declines with increasing age, but that the fall was greater from 20 to 50 years than 50 to 80 years in women, whereas the reverse was true in men.

Lower plasma protein-binding of thyroxine may be an important factor in animals exhibiting reduced thyroxine levels with age (Chen and Walfish, 1979). Yousef and Luick (1971) also suggest that greater thyroidal activity in younger rats

may be a response to increased protein turnover in younger animals. Hollis (1968) found no changes in protein-bound iodine with increasing age in humans, and attributed this to a balance between the lower degradation of protein-bound iodine associated with a lower basal metabolic rate and the lower rate of production of protein-bound iodine in old age.

The population animals of the first Preliminary Investigation were considerably older than the controls, but both male and female population mice had significantly ($P < 0.028$) lower mean serum thyroxine levels than their respective controls (Appendix A). When the study was repeated to determine the mean serum thyroxine concentrations of similarly aged (approximately 120 days) animals, the population males were found to have a significantly ($P < 0.03$) lower mean serum thyroxine concentration than their controls and population female values tended ($P < 0.1$) to be lower than their controls (Tables 2 and 3). The reproductive organs of both male and female inhibited population animals of Experiment Two were highly significantly ($P < 0.001$) smaller than their respective controls, clearly indicating the drastic nature of the reproductive inhibition present in these animals with low serum thyroxine concentrations.

The significant reduction in serum thyroxine observed in population males is only the second clear-cut difference observed in the endocrine status between reproductively inhibited population deermice and controls. The first difference is the significant elevation of serum corticosterone in

population animals (Bradley and Terman, 1981b; Sung, et al., 1977). But, whether this present observation of reduced serum thyroxine is of significance to the mechanism of reproductive inhibition is uncertain. The report by Brown-Grant, et al., (1954b) that thyroidal I^{131} release in rabbits was inhibited by chronic stress could be used to support the speculation that population "stress" in deermice induces a primary thyroxine reduction that is not related to the adrenal response. Fortier, et al., (1970) reported that stress-induced depression of plasma TSH levels was caused by the inhibition of release and synthesis of TSH rather than its accelerated disappearance. Eisenbud (1975) also reported that stress may tend to increase the severity of hypothyroidism in humans by increasing thyroxine turnover rate. On the other hand, the reports of Christian (1964, 1971a, 1978) and Bronson, et al., (1973) that increased density, aggressive interactions, and chronic stress result in hyperactivity of the pituitary-adrenal axis, and that the inhibitory effects of chronic stress are most likely via increased secretion of ACTH and adrenocortical steroids remain well established and convincing. Other research has shown that in laboratory populations of deermice corticosterone levels were significantly higher, relative adrenal weights of females were larger, and reproductive organs significantly smaller than those in control animals (Bradley and Terman, 1981a,b; Sung, et al., 1977). The question is whether the pituitary-adrenal mechanism is the primary cause of reproductive disruption, or,

whether stress disruption of the pituitary-thyroid axis may be the prime, or co-equal, cause of the inhibition.

It has been suggested that ACTH in female mice may act by negative feedback on the hypothalamus or central nervous system to suppress gonadotrophin secretion, and that increased adrenal secretions of androgen and progesterone may augment this effect. A direct inhibitory influence of ACTH on the gonads is also possible (Christian, 1964, 1978). In addition to its possible direct inhibitory influence on reproductive function, hyperactivity of the pituitary-adrenal axis might also be a primary cause of reproductive inhibition via suppression of the pituitary-thyroid axis. In a report by Fredrickson, et al., (1952) it was found that massive doses of cortisone caused a decrease in the percentage of I^{131} accumulated in the thyroid gland and resulted in a significant fall in the level of serum protein-bound iodine in euthyroid patients. Melander and Rerup (1968) argued that the inhibitory effects of corticosteroids on thyroid function in mice is through its promotion of the elimination of inorganic iodine from the blood. The bulk of evidence, however, favors the view that thyroid inhibition occurs through the suppression of pituitary TSH secretion by ACTH and glucocorticoids (Alger and Boccabella, 1968; Brown-Grant, et al., 1954a,b,c; Brown-Grant, 1955; Harris, 1955; Pamenter and Hedge, 1980; Tolis, et al., 1974). However, any repressive effects on thyroid secretion through increased activity of the pituitary-adrenal axis may be confounded by decreased

secretion of the pituitary and adrenal glands associated with thyroid dysfunction. An experimental study on rats (Johansson and Jönsson, 1971) found degenerative changes in the adrenal cortex to occur after thyroidectomy and thiouracil treatment, and Gaunt, et al., (1970) reported that thyroidectomy resulted in a decrease in the corticosterone secretion rate in rats. Peterson (1958) suggested that there was a homeostatic mechanism mediated through the liver, pituitary, and adrenals which caused decreased synthesis of cortisol in patients with myxedema, and an increased synthesis of cortisol in those with thyrotoxicosis. The reduction in adrenal function with primary hypothyroidism is thought to be due to either an impairment of the corticotrophic function or to an inadequate response of the adrenal cortex to corticotrophin (Havard, et al., 1970; Minozzi, et al., 1973). Research on deermice from populations, however, has not demonstrated such an adrenal regression concomitant with thyroxine reduction. Indeed, the serum corticosterone concentrations in population deermice are elevated (Bradley and Terman, 1981b).

The lower thyroxine levels of reproductively inhibited population animals in comparison to controls in the present study could have directly contributed to the small size of their reproductive organs. Hypothyroidism in various young and adult animals has been found to significantly inhibit their reproductive organ weights and cause a regression in the activity of their gonads. Del Rio, et al., (1979) showed

that epididymal connective tissue and vascularization in rats diminished with a 90% reduction in serum levels of triiodothyronine and thyroxine. The inhibitory effects of thyroidectomy on reproductive organ weight include: a reduction in ovarian weight in rats (Jones and Tracy, 1974); decreases in the weights of ovaries, uteri, and vaginas in gerbils (Dixit, et al., 1976); suppression of the number of ova in rats (Hagino, 1971); and a decline in the weights of the seminal vesicles and ventral prostates of male rats (Kalland, et al., 1978). In other reports, thiouracil treatment resulted in the arrest of interstitial cell development in the testes of rabbits (Maqsood, 1951) and rats (Amin and el-Sheikh, 1977), and caused statistically significant decreases in the weights of the testes and seminal vesicles of male mice (Maqsood and Reineke, 1950). Furthermore, the histological appearance of gonads from population deermice (Bradley and Terman, 1981b) strongly resembles the histological description of immature gonads from hypothyroid animals (Amin and el-Sheikh, 1977; Dixit, et al., 1976; Maqsood, 1951).

In addition to its inhibitory effects on reproductive organ weight, hypothyroidism has been found to disrupt reproductive function in a variety of other ways. Other reproductive processes inhibited by thyroid dysfunction include arresting of libido in male rabbits (Maqsood, 1951); decline in testicular metabolism in thyroidectomized rats (Panda and Jindal, 1980); atrophic ovaries with arrested follicular development, and uterine regressions in gerbils (Dixit, et al.,

1976); a decrease in the number of ovulations and increases in the number of unruptured follicles in rabbits (Chu, 1944b); a reduction in the percent of fertile matings, and number of young born live in guinea pigs (Peterson, et al., 1952); lengthening of 46% of the gestations in female mice (Bruce and Sloviter, 1957); pregnancy disturbance, and intermittent parturition in rabbits (Chu, 1944a); changes in rat placental morphology and histology which may contribute to miscarriages, fetus resorption, or premature births (VanWynsberghe and Kiaie, 1980); and a reduction in the mean body weights and numbers of young brought to term in rats (Hendrich, et al., 1976; Krohn and White, 1950).

The fact that altered levels of thyroid hormone affects reproductive status is quite clearly documented. In humans, for example, the relationship of thyroid disorders to altered menstrual patterns is well known (Goldsmith, et al., 1952; Rosenberg, 1969). In addition, Krohn and White (1950) report that after thiouracil treatment the estrous cycles of female albino rats become longer and more variable in length, and Bakke, et al., (1970) also found that perinatal hypothyroidism produced by PTU treatment caused a delay in vaginal opening (puberty) in the rat. Likewise, a reduction in the percent of guinea pigs in estrous (Peterson, et al., 1952), constant vaginal diestrus in gerbils (Dixit, et al., 1976) and prolongation of the estrous cycles in female mice (Bruce and Sloviter, 1957) have been associated with thyroid dysfunction. Rat pups of mothers treated in late pregnancy

with PTU had inhibited gonadal development, and the day of vaginal opening and onset of estrous in females was delayed (Kikuyama, et al., 1974). Walker and Cooper (1978) suggest that constant estrus in the rat may be the result of positive functional changes in the thyroid axis. An indication that insufficient levels of thyroid hormone may also be inhibitory to proper lactation was given by Lorscheider and Reineke (1972), who found evidence for a greater demand of thyroid hormone during lactation in rats. Moreover, uterine metabolism in the normal female rat and the sensitivity and response of the uterus to estrogen is affected by hypo-hyperthyroid conditions (Gardner, et al., 1978; Ruh, et al., 1970; Schultze and Noonan, 1970). Thyroidectomy or thiouracil treatment have been found to interfere with the normal process of spermatogenesis in male rats (Amin and el-Sheikh, 1977) and in male rabbits (Maqsood, 1951).

The precise manner in which thyroid hormone affects reproduction is unclear. Thyroid deficiency may directly influence reproductive processes by disrupting protein metabolism or by an inability of the reproductive tissues to respond to secretions of the pituitary in the absence of thyroid hormone (Turner and Bagnara, 1976). Further, thyroid dysfunction might indirectly inhibit reproductive function by altering the secretions of the pituitary. Studies indicate that serum levels of GH are affected by serum thyroxine concentrations. In humans and rats, thyroidectomy is known to decrease the amount of GH in the pituitary and serum, and the

administration of exogenous thyroxine increases their levels of GH (Coiro, et al., 1979; Hervás, et al., 1975; Minozzi, et al., 1973; Solomon and Greep, 1959). The metabolic clearance rates of the gonadotrophins may not be altered by changes in the status of the thyroid, but the thyroid may influence their synthesis or their secretions by acting directly on the hypothalamo-pituitary axis (Freeman, et al., 1975; Larochelle and Freeman, 1974; Wang, et al., 1980). Bruni, et al., (1975) found that at 30 days after thyro-parathyroidectomy the release of LH and FSH in rats with intact gonads was significantly reduced, and that replacement doses of thyroxine were able to restore their release to normal. Wong, et al., (1980) reports of reduced pituitary concentrations of prolactin and FSH and a noticeable but statistically insignificant decline in LH in rats treated with PTU. In addition, Vilchez-Martinez (1973) showed lower pituitary gonadatrophin concentrations in I^{131} treated rats. Chu (1944b), on the other hand, discovered higher FSH production and diminished pituitary levels of LH in thyroidectomized rabbits and, likewise, Reineke and Soliman (1953) report results indicating that hypothyroidism favors follicular growth whereas hyperthyroidism favors luteinization of the mouse ovary. Although progesterone levels are similar in control and population female deermice (Albertson, et al., 1975), inhibited population females have lower LH concentrations and reproductively inhibited males and females show higher FSH concentrations than controls (Bradley and Terman, 1981a). Based on findings that

excessive FSH levels in female rabbits caused serum estrogen to rise to concentrations which disturbed the course of their pregnancies, Chu (1944b) suggests that increased levels of FSH and decreased levels of LH may be inhibitory to reproduction. Low thyroxine levels in population deermice might, thus, disrupt their reproductive functions by causing FSH levels to increase and LH levels to decrease.

Upon finding that male population deermice have significantly lower serum thyroxine levels and that population females tend to have lower thyroxine levels than their respective controls, a final investigation was undertaken to test the possibility that exogenous thyroxine could induce the testicular development of inhibited population males. In studies by Maqsood (1950), and Maqsood and Reineke (1950), mild hyperthyroidism produced by physiological doses of L-thyroxine caused precocious sexual development in young male rabbits and rams and produced significant increases in the weights of the testes and seminal vesicles of growing male mice. Eartly and Leblond (1954) also found that daily treatments of three to six ug of thyroxine brought the heart and oxygen consumption rate of young thyroidectomized rats to within the range observed in intact controls. The levels of thyroxine administered to the rats were thus considered as physiological doses, and four ug (physiological) thyroxine treatments daily resulted in significant increases in the weights of the testes of the male rats. In rats, thyroid secretion rates have been reported to be between 2.1 and 5.0

ug per day per 100 g body weight (Reineke and Singh, 1955), and in the mouse, Hurst and Turner (1948) suggest that normal thyroxine secretion rate is on the order of 5.5 ug per 100 g body weight per day. Based on these findings, it was assumed that one ug of thyroxine daily might approximate a physiologically effective dose in deermice weighing approximately 25 g; thus, the administration of one ug of thyroxine per day for 10 days to reproductively inhibited population male deermice should restore them toward a euthyroid or mildly hyperthyroid condition.

A preliminary experiment was, therefore, undertaken in which five males from one population were injected daily with one ug thyroxine and returned to the population enclosure. After 10 days the mean testis and seminal vesicle weights of these five injected males were not significantly different when compared with the respective weights of untreated population males in Experiment Two (Appendix D). Histological studies on the testes of these injected mice, however, showed levels of spermiogenesis in the seminiferous tubules of four of the five animals which are rarely reached in untreated inhibited population mice. An evaluation of the number of spermatids with acrosome formation in a standard transect using the method described by Bradley and Terman (1981a), suggests that a level of development exists in the testes of four of the injected mice that is not statistically different from the control pairs used in that study (Appendix E). This may indicate that the exogenous thyroxine, in fact,

initiated the first stages of testicular activity and development in 80% of the treated population males. The thyroxine injections may have initiated spermiogenesis without affecting testicular size and weight due to the short duration of the treatments. Because sham injected controls were not used, the possibility that daily handling and injecting may have influenced the observed development in the testes cannot be discounted. Nevertheless, based on evidence in the literature discussed earlier that mild thyroxine administration may promote the activity and development of reproductive organs in males, it is suggested that the exogenous thyroxine had a stimulatory effect on the sexual maturation of the hypothyroid reproductively inhibited male population deermice.

Previous studies have found that the morphology and activity of testicular tissue as well as its weight is affected by thyroxine treatment. Physiological doses of thyroprotein for short periods stimulated spermatogenesis in young male mice (Maqsood and Reineke, 1950), and physiological doses of L-thyroxine administered for 40 weeks to four-week old male rabbits caused a greater number and size of testicular interstitial cells to appear, a majority of the seminiferous tubules to contain masses of maturing sperm, and an increase in the numbers and activity of spermatogenic cells (Maqsood, 1951). The induction of a mild hyperthyroid state in male rats, by daily injections of 2.5 to 5.0 ug L-thyroxine, from weaning to 82 days of age, resulted in an increase in the

size and number of testicular spermatogenic and interstitial cells (Amin and el-Sheikh, 1977).

Research showing pituitary and serum gonadotrophin levels responding to thyroxine administration further indicate that reproductive organs are influenced by exogenous thyroxine. Amin and el-Sheikh (1977) report that the stimulatory effects of induced hyperthyroidism (2.5 and 5.0 ug L-thyroxine injections) on the development of the testes in rats were associated with increases in the FSH and LH gonadotrophs of the pituitary. Moreover, exogenous thyroxine and triiodothyronine were reported to stimulate increases in pituitary concentrations of prolactin, FSH, and LH of adult rats which were previously reduced with PTU (Wong, et al., 1980), while Aranda, et al., (1976) found that a single dose of thyroxine (1.75 ug) in thyroidectomized rats induced rapid and intense increases in the pituitary LH concentrations, making them like controls. Previous studies, however, have produced conflicting reports on the effects of induced hyperthyroidism on serum levels of LH and FSH in rats. Hendrich, et al., (1976) found that mild hyperthyroidism in the rat did not alter its serum gonadotrophin levels, while Bruni, et al., (1975) report thyroxine treatment to increase serum gonadotrophin levels. Reasons for the discrepancies in results could be due to the effects of varying doses and durations of treatments.

The present study has shown that reproductively inhibited population males have a significantly lower mean

serum thyroxine concentration than controls, and that inhibited population females tend to have lower levels than controls. Population animals of both sexes also had highly significantly lighter reproductive organs than their respective controls, which is suggestive of drastic reproductive inhibition. Based on findings from other investigations that thyroid deficiency is disruptive to reproductive function in many mammalian species and data from the present study, it can be suggested that lower serum concentrations of thyroxine in reproductively inhibited population deermice may be a factor contributing to their reproductive inhibition. However, further studies are needed to determine the extent and degree of the contribution of thyroid activity to the endocrine mechanism of reproductive inhibition.

APPENDIX A

Serum thyroxine, body weight, and age
in population and control mice from a preliminary study.

Treatments	Age (d)	Body weights (g)	Mean body weight (g) (±SEM)	Serum thyroxine (ug/100 ml)	Mean serum thyroxine (ug/100 ml) (±SEM)
Control Females (n=4)	120	16.3		2.20	
	120	18.8	17.62	3.40	3.70
	118	13.5	±1.78	3.00	±0.86
	118	21.9		6.20	
Control Males (n=4)	120	19.9		5.15	
	120	22.2	19.47	2.80	3.40
	120	18.3	±1.03	3.40	±0.62
	118	17.5		2.25	
Population Females (n=4)	710	12.2		2.10	
	496	12.3	12.67**	1.20	1.50*
	472	12.2	±0.44	1.15	±0.21
	751	14.0		1.55	
Population Males (n=4)	1195	17.4		<1.00	
	1078	15.0	15.37**	<1.00	1.13*
	1006	13.5	±0.80	1.43	±0.10
	1065	15.6		1.12	

*P< 0.028 and **P< 0.05 with respect to the corresponding control value.

APPENDIX B

Age, body weight, ovary and combined uterus and ovary weights, reproductive status, and serum thyroxine in female population mice from a preliminary study.

Age (days)	Body weight (g)	Ovary weight (mg)	Ovary and uterus weight (mg)	Reproductive* status	Serum thyroxine (ug/100 ml)
542	12.6	5.00	15.00	P(n), Pr(n)	2.27
542	13.5	3.00	8.50	P(?), Pr(n)	2.17
617	18.2	4.00	15.50	P(n), Pr(n)	3.20
666	13.1	6.00	13.00	P(n), Pr(n)	3.60
666	15.7	5.00	23.00	P(f), Pr(n)	2.49
900	18.4	12.00	36.00	P(n), Pr(n)	3.30
929	14.9	-	-	P(f), Pr(n)	1.94
929	19.8	-	-	P(f), Pr(n)	2.26
1007	15.1	5.00	32.00	P(f), Pr(n)	4.90
1082	12.6	4.00	11.00	P(n), Pr(n)	1.98
1305	16.9	4.00	11.00	P(n), Pr(n)	4.35
1305	17.6	5.50	42.00	PrL	1.90
1336	16.1	7.00	31.00	P(f), Pr(n)	1.80
1336	16.0	6.00	25.00	P(f), Pr(n)	1.76
1498	17.6	3.00	12.00	P(?), Pr(n)	3.25

*P(f) = vagina infrequently perforate
P(?) = vagina doubtfully ever perforate
P(n) = vagina never perforate
PrL = found pregnant and lactating
Pr(n) = never pregnant or lactating

APPENDIX C

Age, body weight, testis and seminal vesicle weights, reproductive status, and serum thyroxine in male population mice from a preliminary study.

Age (days)	Body weight (g)	Testis weight (mg)	Seminal vesicle weight (mg)	Reproductive* status	Serum thyroxine (ug/100 ml)
700	17.2	122.00	107.00	TS	3.85
748	23.2	290.00	250.00	TS(+)	5.60
748	19.7	137.00	91.00	TS(+)	3.55
1007	18.4	147.00	139.00	TS(f)	2.86
1109	19.7	167.00	120.00	TS(f)	1.75
1165	18.8	212.50	-	TS(f)	4.00
1231	15.3	172.00	79.00	TS(f)	1.88
1242	21.0	121.00	40.00	TS(f)	2.65
1242	18.4	165.00	63.50	TS	3.25
1305	20.2	176.00	116.00	TS(+)	2.16
1366	24.4	49.00	3.50	TS(n)	3.60
1366	17.6	83.00	15.00	-	2.88
1498	18.2	98.50	9.00	TS(+)	2.95

*TS(+) = testes most often scrotal
TS = testes frequently scrotal
TS(f) = testes infrequently scrotal
TS(n) = testes never scrotal

APPENDIX D

Testis and seminal vesicle weights in thyroxine-injected population males compared with testis and seminal vesicle weights in males from Experiment Two. Values are mean ± SEM.

Treatments	Testis weight (mg)	Seminal vesicle weight (mg)
Population Males (Exp. Two) (n=22)	92.78 ±13.50	12.81 ±4.46
Population Males (Treatment) (n=5)	67.90$^{n.s.}$ ±10.98	4.40$^{n.s.}$ ±1.91

$^{n.s.}$ = not significant at $P > 0.1$.

APPENDIX E

Spermatid development in five thyroxine-treated (1 ug/d; 10 days) reproductively -inhibited population males compared with a study by Bradley and Terman, 1981*.

Animal identification	Number of spermatids with acrosomes* (mean ± SEM)	Percent of group total with spermatids showing acrosome formation
Reproductively -inhibited population males treated with thyroxine		
#390	0	
#392	16.4 ± 2.96	
#394	12.4 ± 1.15	80%
#396	6.5 ± 1.21	
#398	11.5 ± 1.12	
Population* inhibited (n=14)	2.1 ± 0.61 [n=7]	50%
Control* (n=27)	11.0 ± 0.32 [n=27]	100%

*See Bradley and Terman (1981) Comp. Biochem. Physiol. 68A:563 to 570.
Numbers in parentheses refer to the number of animals evaluated.
Numbers in brackets refer to the number of animals possessing the structure.

BIBLIOGRAPHY

Albertson, B.D., E.L. Bradley, and C.R. Terman. 1975. Plasma progesterone concentrations in prairie deermice (Peromyscus maniculatus bairdi) from experimental laboratory populations. J. Reprod. Fert. 42:407-414.

Alger, E.A. and A.V. Boccabella. 1968. Failure of adrenalectomy to alter either thyroid activity during the estrous cycle or thyroidal response to exogenous estrogen in the rat. Endocrinology 83:1366-1369.

Amin, S.O. and A.S. el-Sheikh. 1977. Pituitary-testicular function changes in hypo- and hyperthyroid male rats. Acta Anatom. 98:121-129.

Aranda, A., F. Hervás, G. Morreale de Escobar, and F. Escobar del Rey. 1976. Effects of small doses of L-thyroxine and L-triiodothyronine on pituitary LH content of thyroidectomized rats. Acta Endocrinol. 83:726-736.

Azizi, F. 1979. Changes in pituitary and thyroid function with increasing age in young male rats. Am. J. Physiol. 237:E224-E226.

Bakke, J.L., R.J. Gellert, and N.L. Lawrence. 1970. The persistent effects of perinatal hypothyroidism on pituitary, thyroidal, and gonadal functions. J. Lab. Clin. Med. 76:25-33.

Bradley, E.L. and C.R. Terman. 1981a. Studies on the nature of reproductive inhibition in animals from laboratory populations of prairie deermice (Peromyscus maniculatus bairdi): serum LH and FSH concentrations. Comp. Biochem. Physiol. 68A:563-570.

Bradley, E.L. and C.R. Terman. 1981b. A comparison of the adrenal histology, reproductive condition, and serum corticosterone concentrations of prairie deermice (Peromyscus maniculatus bairdi) in captivity. J. Mammal. 62:353-361.

Bradley, E.L. and C.R. Terman. 1981c. Serum testosterone concentrations in male prairie deermice (Peromyscus maniculatus bairdi)from laboratory populations. J. Mammal. (accepted for publication).

Bronson, F.H., M. H. Stetson, and M.E. Stiff. 1973. Serum FSH and LH in male mice following aggressive and nonagressive interaction. Physiol. Behav. 10:369-372.

Brown-Grant, K. 1955. Inhibition of the release of thyroidal radioiodine in the rat by cortisone. Endocrinology 56:607-609.

Brown-Grant, K., G.W. Harris, and S. Reichlin. 1954a. The influence of the adrenal cortex on thyroid activity in the rabbit. J. Physiol. 126:41-51.

Brown-Grant, K., G.W. Harris, and S. Reichlin. 1954b. The effect of emotional and physical stress on thyroid activity in the rabbit. J. Physiol. 126: 29-40.

Brown-Grant, K., C. VonEuler, G.W. Harris, and S. Reichlin. 1954c. The measurement and experimental modification of thyroid activity in the rabbit. J. Physiol. 126:1-28.

Brown-Grant, K., G.W. Harris, and S. Reichlin. 1957. The effect of pituitary stalk section on thyroid function in the rabbit. J. Physiol. 136:364-379.

Bruce, H.M. and H.A. Sloviter. 1957. Effect of destruction of thyroid tissue by radioactive iodine on reproduction in mice. J. Endocrinol. 15:72-82.

Bruni, J.F., S. Marshall, J.A. Dibbet, and J. Meites. 1975. Effects of hyper- and hypothyroidism on serum LH and FSH levels in intact and gonadectomized male and female rats. Endocrinology 97:558-563.

Buchanan, G.C., D.R. Tredway, J.C. Pittard, and T.A. Daane. 1977. Gonadotrophin secretion and hypothyroidism. Obstet. and Gynecol. 50:392-396.

Chan, V., C. Wang, and R.T.T. Yeung. 1978. Pituitary-thyroid response to surgical stress. Acta Endocrinol. 83:490-498.

Chen, H.J. and P.G. Walfish. 1978. Effects of age and ovarian function on the pituitary-thyroid system in female rats. J. Endocrinol. 78:225-232.

Chen, H.J. and P.G. Walfish. 1979. Effects of age and testicular function on the pituitary-thyroid system in male rats. J. Endocrinol. 82:53-59.

Chopra, I.J., D.H. Solomon, G.N. Chua Teco. 1973. T_4: Just a prohormone or a hormone too? J. Clin. Endocrinol. Metab. 36:1050-1057.

Christian, J.J. 1955. Effect of population size on the weights of the reproductive organs of white mice. Am. J. Physiol. 181:477-480.

Christian, J.J. 1960. Adrenocortical and gonadal responses of female mice to increased population density. Proc. Soc. Exp. Biol. Med. 104:330-332.

Christian, J.J. 1963. Endocrine adaptive mechanisms and the physiologic regulation of population growth. In Physiological Mammalogy. (Edited by W.V. Mayer and R.G. VanGelder.) pp. 189-353. Academic Press, New York.

Christian, J.J. 1964. Effect of chronic ACTH treatment on maturation of intact female mice. Endocrinology 74:669-679.

Christian, J.J. 1971a. Population density and fertility in mammals. In The Action of Hormones: Genes to Population. (Edited by P.P. Foá.) pp. 471-499. Charles C. Thomas, Springfield, Ill.

Christian, J.J. 1971b. Population density and reproductive efficiency. Biol. Reprod. 4:248-294.

Christian, J.J. 1978. Neurobehavioral endocrine regulation of small mammal populations. In Population of Small Mammals Under Natural Conditions. (Edited by D.P. Snyder.) pp. 143-158. Pymatuning Laboratory of Ecology Special Publication No. 5.

Christian, J.J. 1980. Endocrine factors in population regulation. In Biosocial Mechanisms and Population Regulation. (Edited by M.N. Cohen, R.S. Malpais, and H.G. Klein.) pp. 55-115. Yale Univ. Press, New Haven.

Christian, J.J. and D.E. Davis. 1964. Endocrines, Behavior, and Population. Science 146:1550-1560.

Christian, J.J., V. Flyger, and D.E. Davis. 1960. Factors in mass mortality of Sika deer. Chesapeake Sci. 1:79-95.

Chu, J.P. 1944a. The influence of the thyroid on pregnancy and parturition in the rabbit. J. Endocrinol. 4:109-114.

Chu, J.P. 1944b. Influence of the thyroid gland on pituitary gonadotrophic activity in the rabbit. Endocrinology 34:90-102.

Coiro, V., L.E. Braverman, D. Christianson, S. Fang, and H.M. Goodman. 1979. Effect of hypothyroidism and thyroxine replacement on growth hormone in the rat. Endocrinology 105:641-646.

Contopoulos, A.N., M.E. Simpson, and A.A. Koneff. 1958. Pituitary function in the thyroidectomized rat. Endocrinology 63:642-653.

Coppes, J.C., Jr. 1980. The role of the pituitary-adrenal axis in the reproductive physiology of male prairie deermice (Peromyscus maniculatus bairdi). Masters' Thesis, Dept. of Biology, College of William and Mary in Virginia.

DeCostre P., U. Buhler, L.J. DeGroot, and S. Refetoff. 1971. Diurnal rhythm in total serum thyroxine levels. Metabolism 20:782-791.

Del Rio, A.G., C.L. Valdez Toledo, and M.C. Quiros. 1979. Thyroid gland and epididymal function in rats - histological study. Arch. Androl. 3:19-22.

Dewhurst, K.E., D.J. el-Kabir, and G.W. Harris. 1968. A review of the effect of stress on the activity of the central nervous-pituitary-thyroid axis in animals and man. Confin. Neurol. 30:161-196.

Dixit, V.P., M. Arya, and N.K. Lohiya. 1976. The effect of hypothyroidism on the female genital tract of gerbils. (Meriones hurrianae Jerdon). Acta Biol. Med. Germ. 35:205-211.

Döhler, K.D., K. Gärtner, A. von zur Mühlen, and U. Döhler. 1977. Activation of anterior pituitary, thyroid, and adrenal gland in rats after disturbance stress. Acta Endocrinol. 86:489-497.

Ducommun, P., E. Sakiz, and R. Guillamin. 1966. Lability of plasma TSH levels in the rat in response to non-specific exteroceptive stimuli. Proc. Soc. Exp. Biol. 121:921-923.

Dunn, J.D., A. Arimura, and L.E. Scheving. 1972. Effect of stress on circadian periodicity in serum LH and prolactin concentration. Endocrinology 90:29-33.

Dunn, J.D., M. Hess, and D.C. Johnson. 1976. Effect of thyroidectomy on rhythmic gonadotropin release. Proc. Soc. Exp. Biol. Med. 151:22-27.

Eartly, H. and C.P. Leblond. 1954. Identification of the effects of thyroxine mediated by the hypophysis. Endocrinology 54:249-271.

Eisenbud, E. 1975. Hypothyroidism and stress, Letter. JAMA 231:1339.

Fenske, M. and W. Wuttke. 1977. Development of stress-induced pituitary prolactin and TSH release in male rats. Acta Endocrinol. 85:729-735.

Fleischer, A.S., D.R. Rudman, N.S. Payne, and G.T. Tindall. 1978. Hypothalamic hypothyroidism and hypogonadism in prolonged traumatic coma. J. Neurosurg. 49:650-657.

Fortier, C., A. Delgado, P. Ducommun, S. Ducommun, A. Dupont, M. Jobin, J. Kraicer, B. MacIntosh-Hairdt, H. Marceau, P. Mialhe, C. Mialhe-Voloss, C. Rerup, and G.P. VanRees. 1970. Functional interrelationships between the adeno-hypophysis, thyroid, adrenal cortex, and gonads. Can. Med. Assoc. J. 103:864-874.

Fredrickson, D.S., P.H. Forsham, and G.W. Thorn. 1952. The effect of massive cortisone therapy on measurements of thyroid function. J. Clin. Endocrinol. 12:541-552.

Freeman, M.E., F.T. LaRochelle, and R.B. Moore. 1975. Thyroid hormone regulation of the pulsatile discharges of LH in ovariectomized rats. Endocrinology 97: 738-743.

Gardner, R.M., J.L. Kirkland, J.S. Ireland, and G.M. Stancel. 1978. Regulation of the uterine response to estrogen by thyroid hormone. Endocrinology 103: 1164-1172.

Gaunt, R., E. Gisoldi, N. Smith, B.G. Steinetz, and J.J. Chart. 1970. Effect of thyroidectomy and aminoglutethimide on adrenal function in rats. Endocrinology 87:1088-1090.

Goldsmith, R.E., S.H. Sturgis, J. Lerman, and J.B. Stanbury. 1952. The menstrual pattern in thyroid disease. J. Clin. Endocrinol. Metab. 12:846-855.

Hagino, N. 1971. Influence of hypothyroid state on ovulation in rats. Endocrinology 88:1332-1336.

Harris, G.W. 1955. The reciprocal relationship between the thyroid and adrenocortical responses to stress. CIBA Foundation Colloq. on Endocrinol. 8:531-550.

Havard, C.W.H., V.F. Saldanha, R. Bird, and R. Gardner. 1970. Adrenal function in hypothyroidism. British Med. J. 1:337-339.

Heinen, E., J. Herrmann, and H.L. Krüskemper. 1981. Influence of old age on serum and liver thyroid hormone concentrations in the rat. Acta Endocrinol. 96, Supplementum 240:17.

Hendrich, C.E., S.P. Porterfield, J. Henderson, and V.A. Galton. 1976. A comparison of the effects of altered thyroid and parathyroid function on reproduction in the rat. Horm. Metab. Res. 8:220-226.

Hervás, F., G. Morreale de Escobar, and F. Escobar del Rey. 1975. Rapid effects of single small doses of L-thyroxine and triiodo-L-thyronine on growth hormone, as studied in the rat by radioimmunoassay. Endocrinology 97:91-101.

Hoar, R.M., R.W. Goy, and W.C. Young. 1957. Loci of action of thyroid hormone on reproduction in the female guinea pig. Endocrinology 60:337-346.

Hollis, W.C. 1968. The aged thyroid gland. Geriatrics 23:124-131.

Hurst, V. and C.W. Turner. 1948. The thyroid secretion in the mouse and its relation to various physiological processes. Missouri Agr. Expt. Sta. Res. Bull. 417.

Hwang, J.C., P.H. Li, and W.C.M. Wan. 1974. Effect of induced hypothyroidism on pituitary luteinizing hormone (LH) concentration in female rats. J. Formosan Med. Assoc. 73:227-231.

Johansson, H. and L.E. Jönsson. 1971. Relationship between thyroid and adrenal glands in hypothyroidism. Acta Chir. Scand. 137:59-66.

Jones, R.C. and J. Tracy. 1974. Effects of thyroidectomy on ovarian compensatory hypertrophy in rats. Experientia 30:432-433.

Jordan, D., B. Rousset, F. Perrin, M. Fournier, and J. Orgiazzi. 1980. Evidence for circadian variations in serum TSH, T_3 and T_4 in the rat. Endocrinology 107:1245-1248.

Kalland, G.A., A. Vera, M. Peterson, and R.S. Swerdloff. 1978. Reproductive hormonal axis of the male rat in experimental hypothyroidism. Endocrinology 102: 476-484.

Kenny, F.M., N. Iturzaeta, C. Preeyasombat, F.H. Taylor, and C.J. Migeon. 1967. Cortisol production rate. VII. Hypothyroidism and hyperthyroidism in infants and children. J. Clin. Endocrinol. Metab. 27: 1616-1622.

Kikuyama, S., H. Nagasawa, R. Yanai, and K. Yamanouchi. 1974. Effect of perinatal hypothyroidism on pituitary secretion of growth hormone and prolactin in rats. J. Endocrinol. 62:213-223.

Kirkegaard, C., T. Friis, and K. Siersboek-Nielsen. 1974. Measurements of serum triiodothyronine by radioimmunoassay. Acta Endocrinol. 77:71-81.

Knigge, K.M., C.H. Penrod, and W.J. Schindler. 1959. In vitro and in vivo adrenal corticosteroid secretion following stress. Am. J. Physiol. 196: 579-582.

Krohn, P.L. and H.C. White. 1950. The effect of hypothyroidism on reproduction in the female albino rat. J. Endocrinol. 6:375-385.

Kruhlich, L., E. Hefco, P. Illner, and C.B. Read. 1974. The effects of acute stress on the secretion of LH, FSH, prolactin, and GH in the normal rat, with comments on their statistical evaluation. Neuroendocrinol. 16:293-311.

Laird, C.W. and R. R. Fox. 1970. Diurnal variations in rabbits: biochemical indicators of thyroid function. Life Sciences 9:191-202.

Langer, P. and B. Lichardus. 1969. Thyroid function and its fluctuations during and after short-term stress and dexamethasone administration in rats. Neuroendocrinol. 4:112-121.

LaRochelle, F.T. and M.E. Freeman. 1974. Superimposition of thyroid hormone regulation on gonadotropin secretion. Endocrinology 96:378-387.

Levin, M.E. and W.H. Daughaday. 1955. The influence of the thyroid on adrenocortical function. J. Clin. Endocrinol. 15:1499-1511.

Lorscheider, F.L. and E.P. Reineke. 1972. Thyroid hormone secretion rate in the lactating rat. J. Reprod. Fert. 30:269-279.

Maqsood, M. 1950. Role of the thyroid in sexual development in the male. Nature 166:692.

Maqsood, M. 1951. Influence of thyroid status on spermatogenesis. Science 114:693-694.

Maqsood, M. and E.P. Reineke. 1950. Influence of environmental temperatures and thyroid status on sexual development in male mouse. Am. J. Physiol. 162:24-30.

Martin, M.M., D.H. Mintz, and H. Tamagaki. 1963. Effect of altered thyroid function upon steroid circadian rhythms in man. J. Clin. Endocrinol. 23:242-247.

Mason, J.W., J.V. Brady, W.W. Tolson, J.A. Robinson, E.D. Taylor, and E.H. Mougey. 1961. Multiple and concurrent hormonal responses associated with psychological stress. Program, 43rd meeting Endocrine Society, New York, 83.

McConnon, J., V.V. Row, and R. Volpé. 1971. Simultaneous comparative studies of thyroxine and triiodothyronine distribution and disposal rates. J. Endocrinol. 51: 17-30.

Melander, A. and C. Rerup. 1968. Studies on the thyroid activity in the mouse. I. The effects of thyroxine, corticosteroids, hypophysectomy, and changes in environmental temperature. Acta Endocrinol. 58:202-214.

Minozzi, M., M. Faggiano, G. Lombardi, C. Carella, T. Criscuolo, C. Oliver, and P. Vague. 1973. Somatotrophic and corticotrophic functions in primary hypothyroidism before and after thyroxine treatment. Acta Endocrinol. 74:483-491.

Negro-Vilar, A., E. Dickerman, and J. Meites. 1971. Effects of starvation on hypothalamic FSH-RF and pituitary FSH in male rats. Endocrinology 88:1246-1249.

Nicoloff, J.T., D.A. Fisher, and M.D. Appleman, Jr. 1970. The role of glucocorticoids in the regulation of thyroid function in man. J. Clin. Invest. 49:1922-1929.

Ooka-Souda, S., D.J. Draves, and P.S. Timiras. 1979. Diurnal rhythm of pituitary-thyroid axis in male rats and the effect of adrenalectomy. Endocr. Res. Commun. 6:43-56.

Pallardo, L.F., I. Pericás, and T. Jolin. 1976. Thyroid iodine uptake, thyroid iodine secretion and plasma TSH levels in male rats during the day and night. Acta Endocrinol. 82:517-529.

Pamenter, R.W. and G.A. Hedge. 1980. Inhibition of thyrotropin secretion by physiological levels of corticosterone. Endocrinology 106:162-166.

Panda, J.N. and S.K. Jindal. 1980. Effect of thyroidectomy and adrenalectomy on the physio-chemical status of rat testes. Andrologia 12:444-447.

Parrott, M.W., M.E. Johnston, and P.W. Durbin. 1960. The effects of thyroid and parathyroid deficiency on reproduction in the rat. Endocrinology 67:467-483.

Peterson, R.E. 1958. The influence of the thyroid on adrenal cortical function. J. Clin. Invest. 37: 736-743.

Peterson, R.R., R.C. Webster, B. Rayner, and W.C. Young. 1952. The thyroid and reproductive performance in the adult female guinea pig. Endocrinology 51:504-518.

Reichlin, S. 1966. Control of thyrotropic hormone secretion. In Neuroendocrinology. (Edited by L. Martini and W.F. Ganong.) pp. 445-536. Academic Press, New York.

Reineke, E.P., A.J. Bergman, and C.W. Turner. 1941. Effect of thyroidectomy of young male goats upon certain AP hormones. Endocrinology 29:306-312.

Reineke, E.P. and O.N. Singh. 1955. Estimation of the thyroid secretion rate of the intact rat. Proc. Soc. Exp. Biol. Med. 88:203.

Reineke, E.P. and F.A. Soliman. 1953. Role of thyroid hormone in the reproductive physiology of the female. Iowa State J. Sci. 28:67-82.

Retiene, K., E. Zimmerman, W.J. Schindler, J. Neuenschwander, and H.S. Lipscomb. 1968. A correlative study of endocrine rhythms in rats. Acta Endocrinol. 57:615-622.

Romanov, Y.A. and L.I. Stepanova. 1975. Diurnal rhythms of cell reproduction and of some morphometric indices in the thyroid gland of young rats. Sov. J. Dev. Biol. 5:127-133.

Rosenberg, I.N. 1969. Menstrual instability in thyroid disease. Clin. Obstet. Gynec. 12:755-770.

Rubenstein, H.A., V.P. Butler, Jr., and S.C. Werner. 1973. Progressive decrease in serum T_3 concentrations with human aging: radioimmunoassay following extraction of serum. J. Clin. Endocrinol. Metab. 37:247-253.

Ruh, M.F., T.S. Ruh, and H.M. Klitgaard. 1970. Effect of thyroid states on uterine sensitivity to estrogen. Proc. Soc. Exp. Biol. Med. 134:947-951.

Samel, M. 1968. Thyroid function during postnatal development in the rat. Gen. and Comp. Endocrinol. 10: 229-234.

Schultze, A.B. and J. Noonan. 1970. Thyroxine administration and reproduction in rats. J. Anim. Sci. 30: 774-776.

Smelser, G.G. 1934. Effect of thyroidectomy on testicular function. Anatom. Record 60:53-54.

Smith, P.E. and E.T. Engle. 1930. The influence of thyroidectomy upon the amount of gonadal-stimulating hormone present in the anterior hypophysis. Anatom. Record 45:275.

Solomon, J. and R.O. Greep. 1959. The effect of alterations in thyroid function on the pituitary growth hormone content and acidophil cytology. Endocrinology 65:158-164.

Sung, K.L.P., E.L. Bradley, and C.R. Terman. 1977. Serum corticosterone concentrations in reproductively mature and inhibited deermice (Peromyscus maniculatus bairdi). J. Reprod. Fert. 49:201-206.

Takahashi, K., W.H. Daughaday, and D.M. Kipnis. 1971. Regulation of immuno-reactive growth hormone secretion in male rats. Endocrinology 88:909-917.

Terman, C.R. 1965. A study of population growth and control exhibited in the laboratory by prairie deermice. Ecology 46:890-895.

Terman, C.R. 1966. Population fluctuations of Peromyscus maniculatus and other small mammals as revealed by the North American census of small mammals. Amer. Midland Natur. 76:419-426.

Terman, C.R. 1969. Weights of selected organs of deermice (Peromyscus maniculatus bairdii) from asymptotic laboratory populations. J. Mammal. 50:311-320.

Terman, C.R. 1973. Reproductive inhibition in asymptotic populations of prairie deermice. J. Reprod. Fert., Suppl. 19:457-463.

Terry, L.C., J.O. Willoughby, P. Brazeau, and J.B. Martin. 1976. Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. Science 192:565-567.

Turner, C.D. and J.T. Bagnara. 1976. General Endocrinology. Sixth Edition. pp. 206-207. W.B. Saunders, Philadelphia.

Tolis, G., H.G. Friesen, C.Y. Bowers, and J.M. McKenzie. 1974. Glucocorticoids and thyrotropin releasing hormone (TRH) secretion. Neuroendocrinol. 15:245-248.

Van Wynsberghe, D.M. and V. Kiaie. 1980. Ultrastructure of the rat placenta in altered maternal thyroid states. Physiologist 23, abstract 419:80.

Vigouroux, E. 1976. Dynamic study of post-natal thyroid function in the rat. Acta Endocrinol. 83:752-762.

Vilchez-Martinez, J.A. 1973. Study of the pituitary-testicular axis in hypothyroid adult male rats. J. Reprod. Fert. 35:123-126.

Volpé, R., J. Vale, and M.W. Johnston. 1960. The effects of certain physical and emotional tensions and strains on fluctuations in the level of serum protein-bound iodine. J. Clin. Endocrinol. Metab. 20:415-428.

Walker, R.F. and R.L. Cooper. 1978. Influence of the thyroid gland on ovarian function in the aging rat. Adv. Exp. Med. Biol. 113:111-126.

Wang, P.S., T.C. Liu, and G.L. Jackson. 1980. Effects of thyroidectomy and thyroxine therapy on biosynthesis and release of luteinizing hormone by rat anterior pituitary glands in vitro. Biol. Reprod. 23:752-759.

Weeke, J. and H.J.G. Gunderson. 1978. Circadian and 30-minute variations in serum TSH and thyroid hormones in normal subjects. Acta Endocrinol. 89:659-672.

Wilber, J.F. and R.D. Utiger. 1969. The effect of glucocorticoids on thyrotropin secretion. J. Clin. Invest. 48:2096-2103.

Wong, C.C., K.D. Döhler, and A. von zur Mühlen. 1980. Effects of triiodothyronine, thyroxine, and isopropyldi-iodothyronine on thyroid-stimulating hormone in serum and pituitary gland and on pituitary concentrations of PrL, GH, LH, and FSH in hypothyroid rats. J. Endocrinol. 87:255-263.

Wong, C.C., K.D. Döhler, A. von zur Mühlen, and U. Döhler. 1977. Differential effects of environmental stimuli on the pituitary-thyroid axis of the rat. Acta Endocrinol. 85, Suppl. 212: abstract 265.

Yousef, M.K. and J.R. Luick. 1971. Estimation of thyroxine secretion rate in reindeer, Rangifer tarandus: effects of sex, age, and season. Comp. Biochem. and Physiol. 40:789-795.

VITA

Edgar David Peebles, III

Born in High Point, North Carolina, July 30, 1956. Graduated from Thomas Jefferson High School in Denver, Colorado, June 1974. Received B.S. degree in Marine Science from the University of South Carolina, May 1979. Entered the Biology Graduate Program of the College of William and Mary in Virginia, with a teaching assistantship, August 1979. Presently a candidate for the Master of Arts degree.